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Biological Fate of Tritiated *p*-Biphenylmethyl-(*dl*-tropyl-α-tropinium)-bromide in the Rat[1]

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Absorption, distribution and excretion of tritiated *p*-biphenylmethyl-(*dl*-tropyl-α-tropinium)bromide(^{3}H-BTTB) in the rat were investigated.

After the intravenous injection of ^{3}H-BTTB, half life of the radioactivity in blood was 7 min at first 30 min. Higher radioactivities were incorporated in kidney, liver, pancreas and spleen. Almost negligible radioactivity was seen in amniotic fluid or fetus.

After the intramuscular injection of ^{3}H-BTTB, 6.62% and 60.16% of the radioactivity were excreted in urine and feces, respectively, at 96 hr. The fact may indicate that ^{3}H-BTTB is mainly excreted in bile.

It may be suggested that these behaviors in biological disposition are attributed to have a large molecular weight and to be a quaternary ammonium compound.

p-Biphenylmethyl-(*dl*-tropyl-α-tropinium)bromide (BTTB), a quaternary ammonium derivative of atropine, is an antispasmodic drug. Our previous report described the biological fate of a quaternary ammonium compound of scopolamine.[3] The aim of the present study was to investigate the absorption, distribution, and excretion of BTTB by means of its tritium-labeled compound.

Material and Method

Materials and Animals——Tritium-labeled *p*-biphenylmethyl-(*dl*-tropyl-α-tropinium) bromide (^{3}H-BTTB) was synthesized by the use of catalytic hydrogenation with ^{3}H over Pd-black. 2-Bromo-4-methylbiphenyl (I: 0.2 mmole) was reduced with 2 Ci of ^{3}H gas in the presence of Pd-black, and subsequent reactions as summarized in Chart 1. ^{3}H-BTTB obtained was 1.8 g (yield 82%) and its specific activity was 0.15 mCi/mg (80.55 mCi/mmole). The radiochemical purity was determined by thin-layer chromatography (TLC) with *n*-BuOH–AcOH–H_2O (4:1:5, v/v) as a developing solvent.

Donryu rats, males and pregnant females, were used and their body weight was 200—250 g.

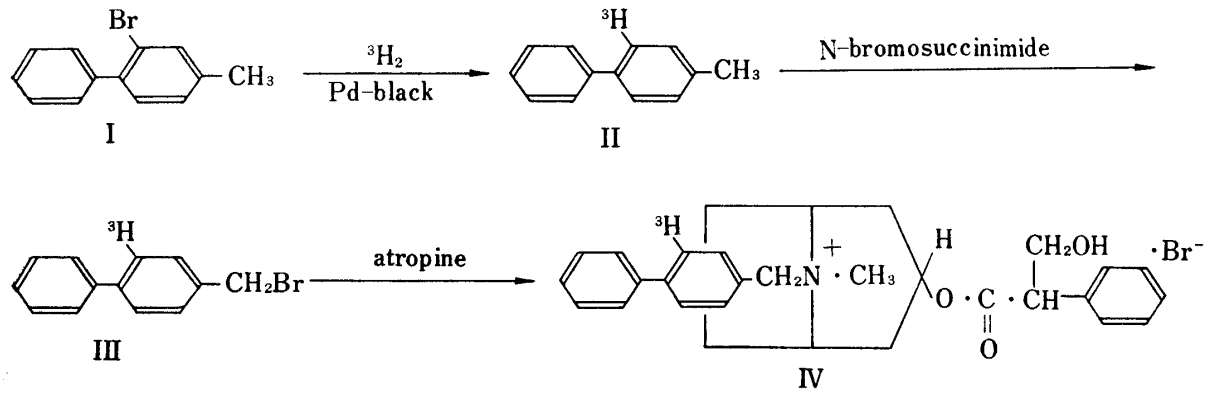

Chart 1

1) A part of this work was presented at the 89th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April, 1969.

2) Location: *Kita-shinjuku 3-20-1, Shinjuku-ku, Tokyo, 160, Japan*: *a*) Present adress: *Funai Pharmaceutical Industries, Ltd. Tsurigane-cho 2-40, Higashi-ku, Osaka.*

3) T. Suga, *Clin. Report* (Japan), 4, 2617 (1970).

Determination of Radioactivity in Blood and Tissues——Blood (10—20 μl) was collected from the tail vein and spotted on strips of filter paper. After allowing to stand for more than 12 hr, dried filter paper was burnt in oxidation flasks and ^3H_2O produced was collected into a dioxane–phosphor scintillator. The radioactivity was counted in an Aloka scintillation counter Model LSC-501.

Tissues were dried over H_2SO_4 in a vacuum desciccator and parts of dried tissue-powder were burnt in the oxidation flasks.

Bile was collected by the method of Watanabe and Ishidate,[4] and its radioactivity was measured in a liquid scintillation counter.

Thin-Layer (TL) Chromatography of Metabolites——Bile was collected after injection of ^{3}H-BTTB and spotted on a silica gel plate of Kieselgel HF_{254}. Ascending development was carried out with *n*-BuOH–AcOH–H_2O (4:1:5, v/v) for about 2 hr. Radioactivity of the chromatogram was measured and recorded by means of TL chromatogram scanner (Aloka Model TLC-2B).

Determination of Serum Protein——Protein concentration was determined by ultraviolet absorption (UV)-method at 280 mμ using bovine serum albumin as a standard.

Result

Changes in Blood Concentration of Radioactivity after Administration of ^{3}H-BTTB

Male Donryu rats were administered ^{3}H-BTTB and blood concentration of radioactivity was measured. After oral administration of 25 mg/kg of ^{3}H-BTTB, peaks of blood concentration were seen at 0.5—3 hr and the radioactivity was 1.2—3.3$\times 10^3$ dpm/ml (Fig. 1a). Apparent peaks of radioactivity were seen during 10 min to 3 hr after intramuscular injection (10 mg/kg) and radioactivity of these peaks was 0.76—1.28$\times 10^4$ dpm/ml (Fig. 1b).

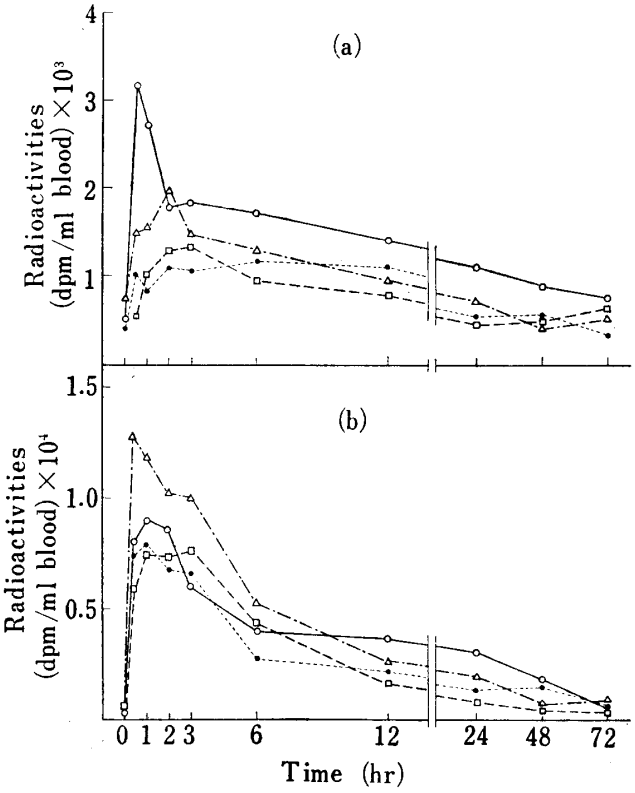

Fig. 1. Changes in Blood Levels of Radioactivity after the Administration of ^{3}H-BTTB in the Rat

(a) oral administration (25 mg/kg): —○—: rat 1; ---△---: rat 2; ------●------: rat 3; --□--: rat 4

(b) intramuscular injection (10 mg/kg): —○—: rat 5; ---△---: rat 6; ------●------: rat 7; --□--: rat 8

As shown in Fig. 2, after intravenous injection (1mg/kg), radioactivity decreased rapidly till 30 min, followed by a slow fall, the half lives being 7 min and 95 min, respectively.

Distribution of Radioactivity after Intravenous Injection of ^{3}H-BTTB in the Rat

Male Donryu rats were intravenously injected with 1 mg/kg (0.15 mCi) of ^{3}H-BTTB. As shown in Table I, radioactivity per gram tissue was high in the

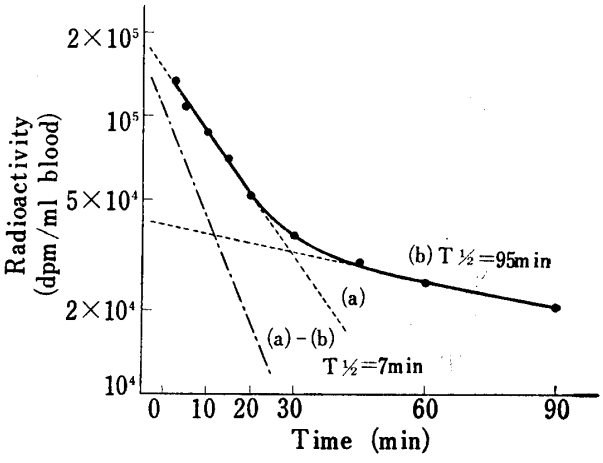

Fig. 2. Changes in Blood Levels of Radioactivity after the Intravenous Injection of ^{3}H-BTTB in the Rat

4) M. Watanabe and M. Ishidate, *Chem. Pharm. Bull.* (Tokyo), **15**, 1461 (1967).

kidney, liver, pancreas, and spleen. Peak of radioactivity was seen at 5 min and the radioactivity decreased with progress of time. At 5 min after injection, 53.8 and 29.7% of radioactivities were seen in whole tissues of liver and kidney, respectively.

Table II shows the distribution of radioactivity in rat at 15 days of gestation, injected with 1 mg (0.15 mCi)/kg of ^{3}H-BTTB. The highest radioactivity was found in the liver and kidney of pregnant rats, and negligible activity was seen in the amniotic fluid and fetus. In the newborn rats (1-day-old), 57.9 and 21.1% of total radioactivity in the whole body were found in the liver and kidney, respectively.

Table I. Distribution of the Radioactivity after the Intravenous Injection of ^{3}H-BTTB in the Rat (Dose: 1 mg/kg, 0.15 mCi/mg)

Tissue	Radioactivities								
	5 min			24 hr			7 days		
	(A)	(B)	(C)	(A)	(B)	(C)	(A)	(B)	(C)
Liver	40.670	430.662	53.8	2.216	46.824	4.84	0.288	2.883	0.32
Kidney	119.225	237.320	29.7	10.513	22.318	2.31	1.487	3.217	0.35
Gastric wall	4.780	4.772	0.60	1.829	2.196	0.22	0.142	0.172	0.02
Lung	1.577	1.183	0.15	0.448	0.550	0.05	0.191	0.194	0.02
Spleen	2.560	1.083	0.14	0.492	0.254	0.02	0.126	0.048	—
Testis	0.326	0.756	0.09	0.121	0.293	0.03	0.067	0.155	0.02
Heart	1.016	0.737	0.09	0.565	0.453	0.04	0.414	0.336	0.03
Brain	0.192	0.286	0.03	0.036	0.056	—	0.013	0.023	—
Small intestine	5.938			0.544			0.081		
Fat	0.322			0.042			0.066		
Thyroid gland	4.236			1.499			0.955		
Pancreas	15.332			11.523			1.878		
Adrenal gland	2.262			0.721			0.161		

Male rats (Donryu strain) were used and each value indicated the mean of 3 animals. (A) (dpm/g tissue) $\times 10^{-5}$ (B) (dpm/whole tissue) $\times 10^{-5}$ (C)% of dose/whole tissue

Table II. Distribution of the Radioactivity in the Fetus and Tissue of Rats after Intravenous Injection of ^{3}H-BTTB

Tissue	Radioactivities			
	Time after injection			
	5 min	30 min	24 hr	7 days
Serum (dpm/ml)	1.042	0.288	0.044	0.006
Liver (dpm/g)	46.440	19.340	0.084	0.224
Kidney (dpm/g)	146.970	102.290	18.560	2.290
Fetus (dpm/g whole body)	0.064	0.063	0.046	0.019[a)]
Placenta (dpm/g)	7.184	8.459	0.399	—
Amniotic fluid (dpm/ml)	0.008	0.006	0.005	—

Pregnant rats (Donryu strain) were dosed with 1 mg ^{3}H-BTTB/kg (0.15 mCi/mg). Each value indicates the mean of 3 animals.
a) new born rats

Urinary and Fecal Excretion of Radioactivity after Administration of ^{3}H-BTTB in the Rat

After oral administration of 25 mg/kg of ^{3}H-BTTB into male Donryu rats, 71.6 and 1.2% of the radioactivity were respectively excreted in feces and urine at 96 hr (Table III). On the other hand, after intramuscular injection (10 mg/kg), 60.16 and 6.62% were excreted in feces and urine, respectively. These results indicate that this chemical may be mainly excreted into bile rather than in the urine.

After ^{3}H-BTTB was injected intravenously into the rats with a cannulation to the bile duct, about 40% of radioactivity was excreted in bile within first 3 hr and another 10% within next 9 hr (Fig. 3). On the other hand, only 15 and 42% were excreted at 3 and 24 hr, respectively, by intramuscular injection. These results suggest that the diffusion from the muscle to the blood stream is a rate-limiting step in the biliary excretion of this chemical.

Table III. Urinary and Fecal Excretion of Radioactivities after Oral or Intramuscular Administration of ^{3}H-BTTB in the Rat

	% excretion		
	(A) Urine	(B) Feces	(A)+(B)
Oral administration			
24 hr	0.83 (0.71— 0.92)	58.1 (32.1 —78.9)	58.9
48 hr	0.15 (0.14— 0.17)	12.6 (11.6 —28.6)	12.8
72 hr	0.14 (0.13— 0.17)	0.7 (0.1 — 1.8)	0.8
96 hr	0.08 (0.06— 0.09)	0.2 (0.0 — 0.6)	0.3
Total	1.20	71.6	72.8
Intramuscular injection			
24 hr	5.82 (1.00—13.90)	21.20 (10.00—33.60)	27.0
48 hr	0.35 (0.24— 0.45)	22.76 (7.60—44.00)	23.2
72 hr	0.30 (0.22— 0.47)	4.32 (3.10— 5.40)	4.6
96 hr	0.15 (0.11— 0.18)	1.88 (1.70— 2.10)	2.1
Total	6.62	60.16	66.8

Values are means of 5 animals (ranges). Dosages were 25 mg/kg (oral) and 10 mg/kg (intramuscular).

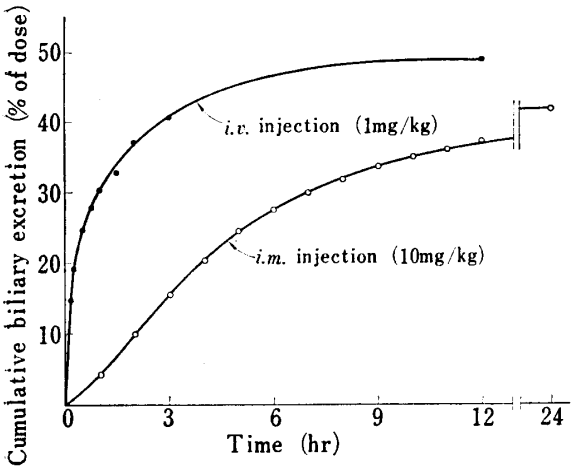

Fig. 3. Cumulative Biliary Excretion of Radioactivity after the Injection of ^{3}H-BTTB in the Rat

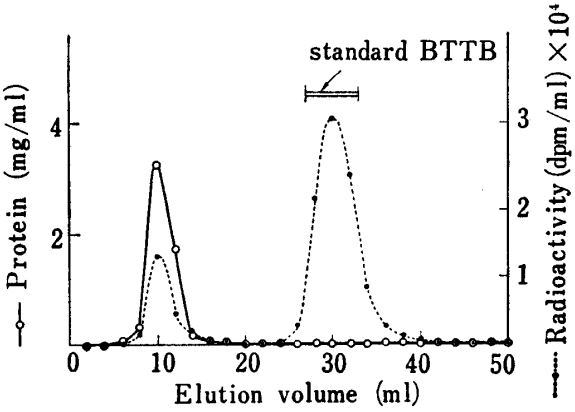

Fig. 4. Gel Filtration of Rat Serum on Sephadex G-25 after the Injection of ^{3}H-BTTB

^{3}H-BTTB was injected intramuscularly to the rat (4 mg/rat, 0.6 mCi). Column was 1.2×10.5 cm and 0.5 ml serum was applied on it. Elution was carried out with 2% NaCl, 2 ml fractions were collected and assayed.

Binding of the Radioactivity to Protein after the Administration of ^{3}H-BTTB

Serum was collected 30 min after 20 mg/kg of ^{3}H-BTTB was injected intramuscularly to the rat, and the serum contained 1.6×10^5 dpm/ml of radioactivity. This serum (0.5 ml) was applied to chromatography over Sephadex G-25 and the column was eluted with 2% NaCl in 0.1 M Tris-HCl buffer, pH 7.4. Fractions were collected in 2 ml portions, and radioactivity and protein concentration of each fraction were measured (Fig. 4).

Serum protein was eluted in the fraction of 8—14 ml, while radioactivities were eluted in the fractions of 8—14 ml and 26—36 ml, separately. Former peak of the radioactivity corresponded to the peak of serum protein and the latter to that of the standard BTTB.

Total radioactivity in serum was 1.06×10^5 dpm/ml, corresponding to 0.032 μg/ml, 5 min after intravenous injection. At this period, protein-bound radioactivity was 1.97×10^4 dpm/ml and the ratio of protein-binding was calculated to be 18.6% from the following equation;

$$\text{ratio of protein-binding (\%)} = \frac{\text{protein-bound radioactivity}}{\text{total radioactivity}} \times 100$$

Fig. 5 shows the ratios of protein-binding of the chemical in tissues. The ratio of protein-binding increased with time and reached 67% at 180 min in serum. In the liver at 5 min after injection, 6.5% of the radioactivity was found to be bound to protein and it reach

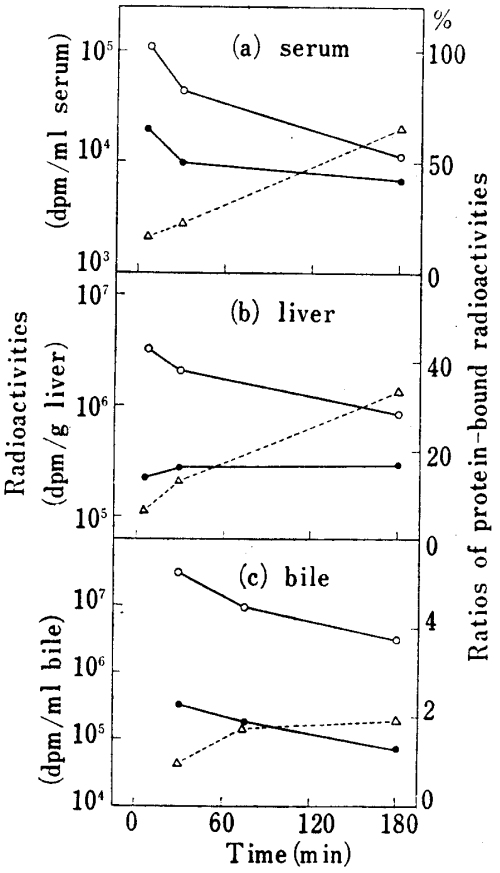

Fig. 5. Changes in Radioactivity and Protein-binding in Tissues after the Intravenous Injection of ^{3}H-BTTB to the Rat (1 mg/kg, 0.15 mCi/mg)

—○—: total radioactivity
—●—: protein-bound radioactivity
—△—: % of total radioactivity

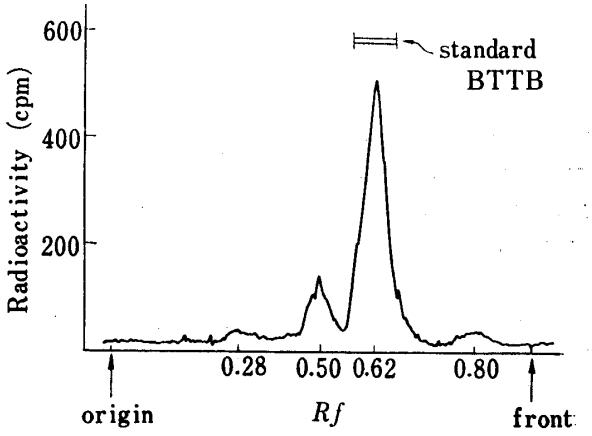

Fig. 6. Thin-Layer Chromatogram of the Bile of Rats Dosed with ^{3}H-BTTB

33.5% at 180 min, less than that in serum. In bile, the ratio of protein-binding was below 2%. These results may indicate that non-protein-bound chemical is involed in the disposition of the drug.

Thin-Layer Chromatography of Metabolites

Figure 6 shows the thin-layer chromatogram of metabolites in bile during the first 30 min after intravenous injection of 1 mg/kg of ^{3}H-BTTB. More than 25% of radioactivity was excreted into bile. Two major peaks (*Rf* 0.50 and 0.62) and two minor peaks (*Rf* 0.28 and 0.80) were detected in the scannogram from TLC. The peak at *Rf* 0.62 coincided with that of the standard BTTB.

Amount of unchanged BTTB in the liver, kidney, feces, and urine was determined by the method of isotope reverse-dilution analysis. As shown in Table IV, 71.2—81.5% in liver, 96.5—101.7% in kidney, 64.5% in feces, and 80.9% in urine were identified as unchanged BTTB.

Discussion

A few reports are seen in the investigation of quaternary ammonium derivatives of atropine. In 1969, Albanus, *et al.*[5] studied the metabolic disposition of methylatropine in

5) L. Albanus, A. Sundwall, and B. Vangbo, *Acta Pharmacol. Toxicol.*, 27, 97 (1969).

Table IV. Analysis of Unchanged BTTB by the Isotope Reverse Dilution Method after the Intravenous Injection of ^{3}H-BTTB

Tissue	Time after the administration	% of radioactivities	
		Mean	Range
Liver	5 min	74.9	68.1— 82.5
	30 min	71.2	67.6— 76.0
	180 min	81.5	87.5— 95.0
Kidney	5 min	96.5	92.0—100.0
	30 min	98.7	91.9—106.0
	180 min	101.7	98.5—106.4
Feces	24 hr	64.5	59.4— 74.8
Urine	24 hr	80.9	78.5— 84.7

Values are means of 3 animals.

mice. They reported that, after subcutaneous injection of this chemical, about 50% of the radioactivity was excreted in the urine within 2 hr and that 40% of total radioactivity in the urine of mice were the unchanged form. In the present work, about 80% of urinary radioactivity was found to be the unchanged form.

Albanus, *et al.*[5] reported that half life of methylatropine in blood of mice was 110 min. After intravenous injection of ^{3}H-BTTB in the present work, two half lives, 7 min and 95 min, were found at first 30 min and thereafter, respectively. These results indicate that the rate of distribution to tissues is significantly rapid. This consideration was supported by the fact that high radioactivity was found in the liver, kidney, and other tissues already at 5 min.

Werner and Schmidt[6] stated that the organs with highest radioactivity were the kidney, liver, intestine, and lung of animals dosed with radioactive scopolamine.

Tamaru, *et al.*[7] reported that azoniaspiro-(3α-benzyloyloxytropane-8,1′-pyrrolidine) chloride was dominantly incorporated into the kidney, liver, and intestine. The present results resemble their results, well.

After ^{3}H-BTTB was injected intramuscularly to the rat, about 60% of the radioactivity was excreted in feces. This result indicates that BTTB is mainly excreted in bile, as supported by the experiment of bile cannulation.

In the mouse, at least 10% of atropine was excreted in bile.[3] Levine and Clark,[8] and Schanker and Solomon[9] reported that procaine amide ethobromide, a quaternary ammonium compound, was excreted in bile by active transport mechanism. Furthermore, Abou-el-Makarem, *et al.*[10] stated that compounds with molecular weight of more than 350 may be excreted selectively in the bile. It may, therefore, be assumed that dominant biliary excretion of ^{3}H-BTTB can be attributed to its large molecular weight (523) and its property of being a quaternary ammonium compound.

Ratio of protein-binding of BTTB in the liver was less than that in blood, and this fact suggests that BTTB is transported in liver in a free form. Furthermore, less than 2% of total radioactivity was found to bind to biliary protein. In the present experiment, a few metabolites were detected in the feces and bile, and further examination is necessary for the identifications of these metabolites.

Acknowledgement The authors are indebted to Dr. Shigeo Baba, Professor of Tokyo College of Pharmacy, for his kind encouragement during the present work.

6) G. Werner and H-L. Schmidt, *Hoppe-Seyler's Z. Physiol. Chem.*, **349**, 677 (1968).
7) S. Tamaru, Y. Mori, and S. Niinobe, *Yakuzaigaku*, **30**, 46 (1969).
8) R.M. Levine, and B.B. Clark, *J. Pharmacol. Exptl. Therap.*, **114**, 63 (1955).
9) L.S. Schanker, and H.M. Solomon, *Am. J. Physiol.*, **204**, 829 (1963).
10) M.M. Abou-el-Makarem, P. Millburn, R.L. Smith, and R.T. Williams, *Biochem. J.*, **105**, 1269 (1967).